

COMPOSITIONS AND METHODS FOR SYNTHESIZING
NUCLEIC ACIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of the filing dates of U.S. Provisional Appl. Nos. 60/408,609, filed September 5, 2002, and 60/427,867, filed November 19, 2002, the disclosures of both of which are incorporated herein by reference in their entireties.

STATEMENT REGARDING FEDERALLY SPONSORED
RESEARCH OR DEVELOPMENT

Not applicable.

~~REFERENCE TO SEQUENCE LISTING/TABLE/COMPUTER PROGRAM
LISTING APPENDIX (submitted on a compact disc and
an incorporation by reference of the material on the compact disc)~~

~~— Not applicable.~~

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] This invention relates to methods and materials useful for nucleic acid synthesis (e.g., polymerase chain reaction-based nucleic acid synthesis).

Related Art

[0003] DNA polymerases (DNAPs) synthesize DNA molecules that are complementary to all or a portion of a nucleic acid template (preferably a DNA template). Upon hybridization of a primer to a DNA template to form a primed template, DNA polymerases can add nucleotides to the 3' hydroxy end

AccuPrime Taq DNA polymerase were used in a typical 50 µl reaction. Primer sets used in development of AccuPrime Taq DNA polymerase system and its applications are listed in Table 2.

TABLE 2

p32D9 149 bp	5'	3'
Forward primer:	ATC CCC CAC CCC CGC ACC (<u>SEQ ID NO:12</u>)	
Reverse primer:	GGG CGC GAG ATG GGC TGC (<u>SEQ ID NO:13</u>)	
Pr1.2 235 bp	5'	3'
Forward primer:	TTG GAG GGG TGG GTG AGT CAA G (<u>SEQ ID NO:14</u>)	
Reverse primer:	GGA GGG GTG GGG GTT AAT GGT TA (<u>SEQ ID NO:15</u>)	
Pr1.3 265 bp	5'	3'
Forward primer:	GCA TCT GGG GCC TGG GAT TTA G (<u>SEQ ID NO:16</u>)	
Reverse primer:	TAC AAG GCA GGC ATC ATG ACT CAC G (<u>SEQ ID NO:17</u>)	
p53 gene 504 bp	5'	3'
Forward primer:	TGC CGT CCC AAG CAA TGG ATT T (<u>SEQ ID NO:18</u>)	
Reverse primer:	CAG GAG AGA TGC TGA GGG TGT GGA (<u>SEQ ID NO:19</u>)	
c-myc gene 822 bp	5'	3'
Forward primer:	CGG TCC ACA AGC TCT CCA CTT G (<u>SEQ ID NO:20</u>)	
Reverse primer:	CTG TTT GAC AAA CCG CAT CCT TG[[c-]] (<u>SEQ ID NO:21</u>)	
c-myc gene 1069 bp	5'	3'
Forward primer:	GGT TTT CGG GGC TTT ATC TAA CTC (<u>SEQ ID NO:22</u>)	
Reverse primer:	GCC TAC CCA ACA CCA CGT CCT (<u>SEQ ID NO:23</u>)	
p53 gene 1587 bp	5'	3'
Forward primer:	GCT GCC GTG TTC CAG TTG CTT TAT C (<u>SEQ ID NO:24</u>)	

	Reverse primer: GCA GCT CGT GGT GAG GCT CCC (<u>SEQ ID NO:25</u>)
p53 gene 1996 bp	5' 3'
Forward primer:	CCT TGG CTT TTG AAA ATA AGC TCC TGA (<u>SEQ ID NO:26</u>)
	Reverse primer: GCA GCT CGT GGT GAG GCT CCC (<u>SEQ ID NO:27</u>)
p53 gene 2108 bp	5' 3'
Forward primer:	GCA GAG ACC TGT GGG AAG CGA AAA (<u>SEQ ID NO:28</u>)
	Reverse primer: GAG AGC TGT GGC AAG CAG GGG A (<u>SEQ ID NO:29</u>)
Rhodopsin gene 3047 bp	5' 3'
Forward primer:	GCC CTA ACT TCT ACG TGC CCT TCT (<u>SEQ ID NO:30</u>)
	Reverse primer: [N]AGG CTT CCA GCG CAC GTC ATT (<u>SEQ ID NO:31</u>)
p53 gene 4356 bp	5' 3'
Forward primer:	CCC CTC CTG GCC CCT GTC AT (<u>SEQ ID NO:32</u>)
	Reverse primer: GTT AGA TGA CTT TGC CCA ACT GTA GGG (<u>SEQ ID NO:33</u>)

[0212] Thermocycling was conducted using either the Perkin Elmer GeneAmp PCR System 9600 or the Perkin Elmer GeneAmp PCR System 2400.

[0213] Standard PCR program:

94°C 2 minutes

35 cycles of

94°C 15 seconds

55°C - 60°C 30 seconds (5 degrees below Tm)

68°C 1 min/kb

Hold at 4°C

[0214] Following the completion of thermocycling, PCR amplification products were mixed with 5 ml of 10x BlueJuice and aliquot (20%, or 10 µl, of total reaction volume per each lane) were analyzed on 0.8% -1.5% agarose gel electrophoresis with an ethidium bromide concentration of 0.5 µg/ml

94°C 15 seconds
55°C-60°C 30 seconds (5 degrees below Tm)
68°C 1 min/kb

Hold at 4°C

[0223] Multiplex PCR. Random designs of primer sets from different genes were selected for multiplex PCR. To determine the optimal conditions, titrations were conducted involving all practical aspects of a standard PCR reaction such as:

- a) DNA template – using 100 ng, 200 ng, and 500 ng.
- b) Enzyme units – with 2 units, 5 units, and 10 units.
- c) dNTP – focusing on 0.1 mM, 0.2 mM, and 0.4 mM final concentrations.
- d) MgCl₂ – centering on, 1.2, 1.5, 1.8, 2, and 2.5 mM final concentrations.
- e) Single Stranded Binding Protein concentration – 200, 400, 600, and 800 ng.

[0224] PCR reactions were prepared on ice in the standard format using 100 ng of K562 genotyping DNA as a template and 2 - 5 units of enzyme in addition to the obvious substitution of each of the variables as outlined above. The primer sets used in multiplex PCR are listed in Table 3.

TABLE 3

#1	Tms1 - 44	5'	3'
	Forward primer:	GGC TGG AGT GCA GTG GTG CAA T <u>(SEQ ID NO:34)</u>	
	Reverse primer:	GGC AGA GGC TAC AGT GAG CCA A <u>(SEQ ID NO:35)</u>	
#2	Thal - 57	5'	3'
	Forward primer:	GGG CAG AGC CAT CTA TTG CTT ACA <u>(SEQ ID NO:36)</u>	
	Reverse primer:	GGT TGC TAG TGA ACA CAG TTG TGT CA <u>(SEQ ID NO:37)</u>	

#3	Hba2 – 67	5'	3'
	Forward primer:	GCA CTC TTC TGG TCC CCA CAG A	<u>(SEQ ID NO:38)</u>
	Reverse primer:	TTG GTC TTG TCG GCA GGA GAC A	<u>(SEQ ID NO:39)</u>
#4	Rgr – 74	5'	3'
	Forward primer:	CCC ACG ATC AAT GCC ATC AAC T	<u>(SEQ ID NO:40)</u>
	Reverse primer:	CGG TGA GAG GCA CTG CCA GAT T	<u>(SEQ ID NO:41)</u>
#5	B-glo-thal – 84	5'	3'
	Forward primer:	GCT CGC TTT CTT GCT GTC CAA T	<u>(SEQ ID NO:42)</u>
	Reverse primer:	GCC CTT CAT AAT ATC CCC CAG TTT	<u>(SEQ ID NO:43)</u>
#6	c-myc – 100	5'	3'
	Forward primer:	GTC CTT CCC CCG CTG GAA AC	<u>(SEQ ID NO:44)</u>
	Reverse primer:	GCA GCA GAG ATC ATC GCG CC	<u>(SEQ ID NO:45)</u>
#7	Zip – 116	5'	3'
	Forward primer:	GTG GGG GTG CTG GGA GTT TGT	<u>(SEQ ID NO:46)</u>
	Reverse primer:	TCG GAC AGA AAC ATG GGT CTG AA	<u>(SEQ ID NO:47)</u>
#8	Csh1 – 135	5'	3'
	Forward primer:	GGT GCT CAG AAC CCC CAC AAT C	<u>(SEQ ID NO:48)</u>
	Reverse primer:	CCT ACC GAC CCC ATT CCA CTC T	<u>(SEQ ID NO:49)</u>
#9	Sub – 153	5'	3'
	Forward primer:	CAC AGA TTT CCA AGG ATG CGC TG	<u>(SEQ ID NO:50)</u>
	Reverse primer:	CGT GCT CTG TTC CAG ACT TG	<u>(SEQ ID NO:51)</u>
#10	Svmt – 170	5'	3'

	Forward primer:	CGT CTG GCG ATT GCT CCA AAT G (<u>SEQ ID</u>)
<u>NO:52)</u>		
	Reverse primer:	GGG CAG TTG TGA TCC ATG AGA A (<u>SEQ ID NO:53</u>)
#11	Olf – 183	5' 3'
	Forward primer:	GGC TTG CAC CAG CTT AGG AAA G (<u>SEQ ID</u>)
<u>NO:54)</u>		
	Reverse primer:	CGT TAG GCA TAA TCA GTG GGA TAG T (<u>SEQ ID</u>)
<u>NO:55)</u>		
#12	P53 – 193	5' 3'
	Forward primer:	GCC TCT GAT TCC TCA CTG ATT GCT CT (<u>SEQ ID</u>)
<u>NO:56)</u>		
	Reverse primer:	TGT CAA CCA CCC TTA ACC CCT CC (<u>SEQ ID NO:57</u>)
#13	Pr 1.2 – 237	5' 3'
	Forward primer:	TTG GAG GGG TGG GTG AGT CAA G (<u>SEQ ID</u>)
<u>NO:58)</u>		
	Reverse primer:	GGA GGG GTG GGG GTT AAT GGT TA (<u>SEQ ID</u>)
<u>NO:59)</u>		
#14	Hmk – 243	5' 3'
	Forward primer:	GGA ACA AGA CAC GGC TGG GTT (<u>SEQ ID</u>)
<u>NO:60)</u>		
	Reverse primer:	AGC AAG GCA GGG CAG GCA A (<u>SEQ ID NO:61</u>)
#15	Rhod – 273	5' 3'
	Forward primer:	CGG TCC CAT TCT CAG GGA ATC T (<u>SEQ ID</u>)
<u>NO:62)</u>		
	Reverse primer:	GCC CAG AGG AAG AAG AAG GAA A (<u>SEQ ID NO:63</u>)
#16	Caafl – 300	5' 3'
	Forward primer:	GCC CCC ACC CAG GTT GGT TTC TA (<u>SEQ ID</u>)
<u>NO:64)</u>		
	Reverse primer:	ATG CCT TCA TCT GGC TCA GTG A (<u>SEQ ID NO:65</u>)
#17	P-450 B – 319	5' 3'
	Forward primer:	GCT CAG CAT GGT GGT GGC ATA A (<u>SEQ ID</u>)
<u>NO:66)</u>		
	Reverse primer:	CCT CAT ACC TTC CCC CCC ATT (<u>SEQ ID NO:67</u>)
#18	S-100 – 360	5' 3'

Forward primer: GAC TAC TCT AGC GAC TGT CCA TCT C (<u>SEQ ID NO:68</u>)		
Reverse primer: GAC AGC CAC CAG ATC CAA TC (<u>SEQ ID NO:69</u>)		
#19	B-cone - 432	5' 3'
	Forward primer:	GGC AGC TTT CAT GGG CAC TGT (<u>SEQ ID NO:70</u>)
Reverse primer: GAC AGG GCT GGA CTG ACA TTT G (<u>SEQ ID NO:71</u>)		
#20	Hbg - 469	5' 3'
	Forward primer:	CTG CTG AAA GAG ATG CGG TGG (<u>SEQ ID NO:72</u>)
Reverse primer: AGG AAA ACA GCC CAA GGG ACA G (<u>SEQ ID NO:73</u>)		

[0225] Standard program for multiplex PCR reactions

94°C 2 minutes

35 cycles

94°C 15 seconds

60°C 30 seconds (5 degrees below Tm)

68°C 1 min/kb

Hold at 4°C

[0226] The PCR products were then analyzed on a 3% horizontal agarose gel with an ethidium bromide concentration of 0.5 µg/ml premixed in 0.5 x TBE. Comparisons were made visually for specificity and yield between the different samples.

[0227] High throughput PCR. Accuprime Taq DNA polymerase was compared with Platinum™ Taq DNA polymerase (Invitrogen Corp.) to examine for improvement in high throughput screening. Standard PCR was performed for 18 cycles of amplification using 2 Units of Accuprime Taq DNA polymerase and 2 Units of Platinum Taq DNA polymerase.

[0228] Transformed cells plated on X-gal/IPTG/Amp plates containing the pUC19 plasmid DNA insert were used as plasmid template for high throughput screening. Mutant colonies were selected with a sterile pipette tip and mixed in the standard PCR reactions. PCR cycling parameters were 94°C

expression in a particular host, depending on the desired expression level. Thus, a single rare codon or a larger percentage (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) of rare codons can be optimized in an SSB gene.

[0371] Attempts to express and purify archaeal SSBs in *E. coli* have met with problems associated with codon bias. For example, SSBs from *Methanococcus jannachii* (MjaSSB) and *Sulfolobus solfataricus* (SsoSSB) are expressed at relatively low levels in BL21(DE3) cells. In addition, SsoSSB co-purifies with shorter peptides most likely truncated proteins arising from premature termination. Present in high enough amounts, the shorter peptides can negate the SsoSSB-mediated PCR improvement.

[0372] The MjaSSB and SsoSSB genes use codons that are rarely used in *E. coli*. For example, in the native MjaSSB and SsoSSB genes AGA or AGG call for arginine, ATA calls for isoleucine, and CTA calls for leucine (Tables 16 and 17; rare codons are underlined). Many of these rare codons occur in tandem pairs or triplets, which may be responsible for the low expression level and/or truncated peptide contaminants.

TABLE 16

<i>Sequence of the native SsoSSB gene</i>
atg gaa gaa aaa gta ggt aat <u>CTA</u> aaa cca aat atg gaa agc gta aat gta acc gta <u>AGA</u> gtt ttg gaa gca agc gaa gca <u>AGA</u> caa <u>ATA</u> cag aca aag aac ggt gtt <u>AGA</u> aca atc agt gag gct att gtt gga gat gaa acg gga <u>AGA</u> gta aag tta aca tta tgg gga aaa cat gca ggc agt <u>ATA</u> aaa gaa ggt caa gtg gta aag <u>ATA</u> gaa aac gcg tgg acc acc gct ttt aag ggt caa gta cag tta aat gct gga agc aaa act aag <u>ATA</u> gct gaa gct tca gaa gat gga ttt cca gaa tca tct caa <u>ATA</u> cca gaa aat aca cca aca gct cct cag caa atg cgt gga gga gga <u>AGA</u> gga ttc cgc ggt ggg gga <u>AGA</u> <u>AGG</u> tat gga <u>AGA</u> <u>AGA</u> ggc ggt <u>AGA</u> <u>AGA</u> caa gaa aac gaa ggt gaa gag gag tga <u>(SEQ ID NO:84)</u>

att aaa gat gaa aac att gaa gct cca gag tat gag CTA
aaa tat tgc aaa att gaa gat att tat aat AGA gat gtt
gac tgg aac gat ATA aat tta ATA gct caa gtt gtt gag
gat tat gga gtt aat gaa att gaa ttt gaa gat aag gtt
AGA aaa gta AGA aat tta ttg tta gaa gat gga act gga
AGA ATA AGG ttg agt tta tgg gat gat ttg gct gaa ATA
gag att aaa gaa gga gat att gta gaa att tta cat gcc
tat gct aag gag AGG gga gat tat ATA gat ttg gtt att
gga aaa tat gga AGA ATA att ATA aat cca gaa ggg gtt
gaa ATA aaa acc aat AGA aag ttt ATA gca gat att gaa
gac gga gaa act gtt gaa gtt AGA ggg gct gta gtt aag
ATA ttg agt gac act ctc ttt ctt tat tta tgc cca aat
tgt AGA aag AGG gtt gta gag att gat gga att tat aac
tgc cct att tgt gga gat gtt gag cca gaa gag att tta
AGA ttg aat ttt gtt gta gat gat ggg act gga act tta
tta tgt AGG gct tat gat AGA AGA gtt gag aag atg tta
aaa atg aat AGG gag gag tta aag aac CTA act ATA gaa
atg gtg gaa gat gaa ATA tta ggg gaa gag ttt gtt ttg
tat gga aat gtt AGA gta gag aat gat gaa tta att atg
gtt gtt AGA AGA gtt aat gat gta gat gtt gag aaa gaa
ATA AGA ATA ttg gag gaa atg gaa taa (SEQ ID NO:85)

[0373] Codon optimization of SsoSSB. To test whether low expression of SsoSSB was related to codon bias, the native gene was transformed into BL21 CodonPlus with supplementary tRNA genes for Arg (AGA, AGG), Ile (AUA) and Leu (CUA) rare codons (Stratagene). When expressed in this host, a SsoSSB was produced at higher levels (compare lanes 12 and lane 13 in Figure 43), and less truncated peptide was present after purification (compare Figures 30 & 31 with Figure 28).

[0374] We replaced the rare codons in the SsoSSB gene with codons common in *E. coli* using “synthetic gene” technology (Stemmer, W. P. et al. (1995) *Gene* 164:49-53). Thus, AGA and AGG were replaced by CGG, CGT, CGA

or CGC; ATA was replaced by ATT or ATC; and CTA was replaced by CTT, CTG, or CTA (Table 18; optimized codons are underlined and in bold italics).

TABLE 18

<i>Codon optimized recombinant SsoSSB gene</i>
atg gaa gaa aaa gta ggt aat <u>ctg</u> aaa cca aat atg gaa agc gta aat gta acc gta <u>cga</u> gtt tt <u>g</u> gaa gca agc gaa gca <u>cgt</u> caa <u>atc</u> cag aca aag aac ggt gtt <u>cgg</u> aca atc agt gag gct att gtt gga gat gaa acg gga <u>cga</u> gta aag tta aca tta tgg gga aaa cat gca ggt agt <u>atc</u> aaa gaa ggt caa gtg gta aag <u>att</u> gaa aac gcg tgg acc acc gct ttt aag ggt caa gta cag tta aat gct gga agc aaa act aag <u>atc</u> gct gaa gct tca gaa gat gga ttt cca gaa tca tct caa <u>att</u> cca gaa aat aca cca aca gct cct cag caa atg cgt gga gga <u>cgc</u> gga ttc cgc ggt ggg gga <u>cgt</u> <u>cgg</u> tat gga <u>cga</u> <u>cgt</u> ggt ggt <u>cgc</u> <u>cgg</u> caa gaa aac gaa ggt gaa gag gag tga <u>(SEQ ID NO:86)</u>

[0375] To make a codon optimized SsoSSB gene, 21 overlapping primers were used (Table 19). The primers were mixed together in equal amounts (approximately 4.5 uM) in a PCR reaction without template DNA. PCR was performed using Taq Hi-FI Supermix (Invitrogen Corp.). A thermocycler was programmed for 20 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 30. An aliquot of this PCR reaction (2 µl or 1/25 of the volume) was added to a second PCR reaction with two 2 anchor primers that anneal at the 5' and 3' ends of the reassembled gene (Table 19). These primers also add a NdeI site to the 5' end and a BamHI site to the 3' end of the gene. After 2 more rounds of PCR using the parameters set out above, a discrete product of about 450 base pairs was obtained. The product was excised from an electrophoresis gel, purified, and cloned into pET21a vector at the NdeI and BamHI sites in the multi-cloning site. The resulting clone was sequenced to confirm the sequence.

TABLE 19

Forward Primers	
Sso F1	ATGGAGAAA AACTAGGTA TCTGAAACCA AATATGGAAA GC (SEQ ID NO:87)
Sso F2	GTAATGTAA CGGTACGAGT TTGGGAGCA AGCGAAGCAC GT (SEQ ID NO:88)
Sso F3	CAAATCCAGA CAAAGAACGG TGTTGGACA ATCAGTGAGG CT (SEQ ID NO:89)
Sso F4	ATTGTGGAG ATGAAACGG ACAGGAAAGG TTAACATTAT GG (SEQ ID NO:90)
Sso F5	GGAAAACATG CAGGTAGTAT CAAAGGAAGT AAGTGGTAAA G (SEQ ID NO:91)
Sso F6	ATTGAAAACG CGTGGACCAC CGCTTTAAG GGTCAAGTAC AG (SEQ ID NO:92)
Sso F7	TAAATGCTG GAAGCAAAAC TAAGATCGCT GAAGCTTCAG AA (SEQ ID NO:93)
Sso F8	GATGGATTTC CAGAAATCATC TCAAATTCCA GAAAATACAC CA (SEQ ID NO:94)
Sso F9	ACAGCTCCTC AGCAAATGCG TGGAGGAGGA CGGGGATTCC GC (SEQ ID NO:95)
Sso F10	GGTGGGGAC GTCGGTATGG ACCGACGTGGT GGTGCGGGC AA (SEQ ID NO:96)
Sso F11	GAAAACGAAG AAGGTGAAGA GGAGTGA (SEQ ID NO:97)
Reverse Primers	
Sso R1	TCACTCCTCT TCACCTCTT CGTTTCTTG CCGGCACCA CC (SEQ ID NO:98)

Sso R2	ACGTCGTCCA TACCGACGTC CCCCCACGGCG GAAATCCGCGT CC (SEQ ID NO: 99)
Sso R3	TCCTCACCGC ATTGTGCTGAG GAGCTGTTGG TGATATTTCCT GG (SEQ ID NO:100)
Sso R4	AATTGAGAT GATTCTGGAA ATCCATCTTC TGAAGCTTCA GC (SEQ ID NO:101)
Sso R5	GATCTAGTT TTGCTTCCAG CATTAACTG TACTTGACCC TT (SEQ ID NO:102)
Sso R6	AAAAGGGTG GTCCACGGGT TTCAATCTT TACCACTTGA CC (SEQ ID NO:103)
Sso R7	TTCTTGATA CTACCTGCAT GTTTTCCCCA TAATGTTAAC TT (SEQ ID NO:104)
Sso R8	TACTGTCCCC GTTTCATCTC CAACAATAGC CTCACTGATT GT (SEQ ID NO:105)
Sso R9	CCGAACACCG TTCTTTGTCT GGATTGACG TGCTTCGCT GC (SEQ ID NO:106)
Sso R10	TTCCAAAACT CGTACGGTTA CATTIACGCT TTCCATATT GG (SEQ ID NO:107)
Sso R11	TTTCAGATTAA CCTACTTTT CTTCCTAT (SEQ ID NO:108)
<i>Anchor primers</i>	
Sso F NdeI	AATTCAATATG GAAGAAAAAGT AGGT (SEQ ID NO:109)
Sso R BamHI	GGAAGGATCC TCACTCCCTT CACCTTC (SEQ ID NO:110)

<p><u>CGT</u> <u>ATC</u> aca aac tgt <u>CGC</u> gtt aag acg ttt tat gat <u>CGT</u> gaa gga aat aaa <u>CGG</u> act gat tta gtt gcc aca tta gaa aca gaa gtt att aaa gat gaa aac att gaa gct cca gag tat gag <u>CTG</u> aaa tat tgc aaa att gaa gat att tat aat <u>CGC</u> gat gtt gac tgg aac gat <u>ATA</u> aat tta <u>ATC</u> gct caa gtt gtt gag gat tat gga gtt aat gaa att gaa ttt gaa gat aag gtt <u>CGT</u> aaa gta <u>CGC</u> aat tta ttg tta gaa gat gga act gga <u>CGT ATT</u> <u>CGG</u> ttg agt tta tgg gat gat ttg gct gaa <u>ATT</u> gag att aaa gaa gga gat att gta gaa att tta cat gcc tat gct aag gag <u>CGG</u> gga gat tat <u>ATC</u> gat ttg gtt att gga aaa tat gga <u>CGA ATT</u> att <u>ATC</u> aat cca gaa ggg gtt gaa <u>ATC</u> aaa acc aat <u>CGT</u> aag ttt <u>ATT</u> gca gat att gaa gac gga gaa act gtt gaa gtt <u>CGC</u> ggg gct gta gtt aag <u>ATC</u> ttg agt gac act ctc ttt ctt tat tta tgc cca aat tgt <u>CGT</u> aag <u>CGG</u> gtt gta gag att gat gga att tat aac tgc cct att tgt gga gat gtt gag cca gaa gag att tta <u>CGA</u> ttg aat ttt gtt gta gat gat ggg act gga act tta tta tgt <u>CGG</u> gct tat gat <u>CGC CGT</u> gtt gag aag atg tta aaa atg aat <u>CGG</u> gag gag tta aag aac <u>CTT</u> act <u>ATC</u> gaa atg gtg gaa gat gaa <u>ATT</u> tta ggg gaa gag ttt gtt ttg tat gga aat gtt <u>CGA</u> gta gag aat gat gaa tta att atg gtt gtt <u>CGT CGC</u> gtt aat gat gta gat gtt gag aaa gaa <u>ATT CGT ATC</u> ttg gag gaa atg gaa taa <u>(SEQ ID</u> <u>NO:111)</u></p>

[0379] The primers identified in Table 21 are used to replace the rare codons in the MjaSSB gene with codons common in *E. coli* using "synthetic gene" technology, as was done for the SsoSSB gene. The forward and reverse primers are about 60 nucleotide long and overlapping at least 15 nucleotides with the neighboring primers.

TABLE 21

Forward Primers	
Mja	F1 ATGGTAGGAG ATTATGAACG TTTAAACAA CTCAAAAAA AGGTGTGCTGA AGCATTGAAT (SEQ ID NO:112)
Mja	F2 GATAAAAAA TTGAGAAAA CGGAGAAC ATTGGAAAG ATGGTCATT ATGATGATT (SEQ ID NO:113)
Mja	F3 AAAATGATG AAGGATTTT ATTAGTGT ATTGAAGAGG GACAGATTG CGTGAGATC (SEQ ID NO:114)
Mja	F4 AAAACATTCA AACGGCGCA TGGAGTTA GGGAAATACA AACGAAATTAC ATTGGGGAT (SEQ ID NO:115)
Mja	F5 GACGATTGG CTGAATTAGA TGAAAAGT GGAGATGTT TAAATTGA ACGCGCACGG (SEQ ID NO:116)
Mja	F6 AGTTCAACAT CTGAAACTAA GATTAAAAAA TTAGAAAAT ATGAAGGAGA ACTTCCAGAG (SEQ ID NO:117)
Mja	F7 AGTCCCTGAA TGACAGCAAC ATTGAGGA GAAGTTATCT CAGCTCTCC ATCAAAAGAA (SEQ ID NO:118)
Mja	F8 TTAAATCAT TTATTGTCG CGATGAGACA GGAAGTATC GCGTACCTT ATGGGATAAT (SEQ ID NO:119)
Mja	F9 TACGTTCGTG TTCCGGCTA TATCAGGAA GTTATTATG GGGTTAGA ATGCACCGCA (SEQ ID NO:120)
Mja	F10 AAAATAGAGA GTGAGAAGT AAATATTGAG GATTAAACAA AATATGAAGA TGGAGAACIG (SEQ ID NO:121)
Mja	F11 AGTAATAAA AAAGCTAGA TTGGATGGA GAGATTGCAA AGGTCAAGA TATTATCTTA (SEQ ID NO:122)
Mja	F12 TTTGGGGG GAAAATGTC TTATGGAA AATATCAAG AAGGGACTT AGTCGTATC (SEQ ID NO:123)
Mja	F13 CGTGAAGGAA ATAACCGAC TGTATTGT GCCACATTAAC AACAGAACT TATTAAGAT (SEQ ID NO:124)
Mja	F14 AAATATGCCA AAATGAGA TATTATAAT CGCGATGTTG ACTGGAACGA TATAAATTAA (SEQ ID NO:125)
Mja	F15 AATGAAATTG AATTCGAGA TAAGGTTGT AAAGTAGGCA ATTATTTGTT AGAAGATGGA (SEQ ID NO:126)
Mja	F16 GATTGGCTG AAATGAGAT TAAGAAGGA GATATTGTAG AAATTTACA TGCCTATGCT (SEQ ID NO:127)
Mja	F17 ATTGGAAAT ATGGACCAAT TATTATCAAT CCAGAAGGGG TTGAATCAA ACCAATCGT (SEQ ID NO:128)
Mja	F18 ACTGTTGAAG TTGGGGGC TTGAGTTAAG ATCTTGAGTG ACACCTCTT TCTTTTATTAA (SEQ ID NO:129)
Mja	F19 ATTGATGGAA TTATAACTG CCCTATTGT GGAGATGTT AGCCAGAAGA GATTTACGA (SEQ ID NO:130)

Mja	F20	ACTTTTATTAT GTCGGGCTTA TGATGCCGT GTTGAGAGA TGTTAAAAT GAATGGGAG (SEQ ID NO:131)
Mja	F21	GAAGATGAAA TTTAGGGG AGAGTTGGT TTGTATGAA ATGTCGAGT AGAGAATGAT (SEQ ID NO:132)
Mja	F22	GATGTAGATG TIGAGAAAGA ATTCTGTATC TTGGAGAAA TGGAAATAA (SEQ ID NO:133)
Reverse Primers		
Mja	R1	TTCATTTT TTATCAATCA TCCGATCTAA TTCCCTCTCA CTAATATTCA ATGCTTCAGC (SEQ ID NO:134)
Mja	R2	TTCTTCATCA TTTTTTCTT CTCCATAAAC TCCATGTTCT TTTGCAATCA TCATTAATGC (SEQ ID NO:135)
Mja	R3	CGGTTGAAT GTTTGATT CAGAGATAC AGTAATACT CCAGTGATCT CAACGCCAAT (SEQ ID NO:136)
Mja	R4	TTCAGCAAA TCGTCCCATA AAGTCATACG AATAGTCTT GACTTATCCG CAATTGTAAT (SEQ ID NO:137)
Mja	R5	TTCAAGTGTG GAACTCACT CTAAATTAT TCGCCATTAA CGTCCCCGTG CGCGTTCAAT (SEQ ID NO:138)
Mja	R6	TGTCAATCCA GGACTCAGCT CACCAATAAT GTAGGTATCT TTAATCTCTG GAAGTTCTCC (SEQ ID NO:139)
Mja	R7	AATAAATGAT TTTAATTTT CAATACTAC ATCAGCACGT TAAATTCTT TGATGGAAAG (SEQ ID NO:140)
Mja	R8	CCGAACACGA ACGTAACTC CAGGACCAAATCGATATCT GTAAAGATTAT CCCATAAGGT (SEQ ID NO:141)
Mja	R9	TTCACTCT ATTTCCTCTC CTTTTTTAA AATCTCTACA TAATTGCGG TGCATTCTAA (SEQ ID NO:142)
Mja	R10	GCTTTTTTA TTACTGATGG CAATAACTCG ACCTTAAACA CTCACCAGTT CTCCATCTTC (SEQ ID NO:143)
Mja	R11	TTTTCCCGC CAAATGAAA CAGGAACCTG ACCAGTGCCG TTATCTAAGA TAATATCTTG (SEQ ID NO:144)
Mja	R12	TTTATTTCCT TCACGATCAT AAAACGTCCT AACGCGACAG TTGTGATAAC GAACTAAGTC (SEQ ID NO:145)
Mja	R13	AATTTCGAA TATTTCAGCT CATACTCTGG AGCTTCATG TTTTCATCTT TAATAACCTC (SEQ ID NO:146)
Mja	R14	AAATTCAATT TCATTAACTC CATAATCCCT AACAAACTTGA GCGATTAAAT TTATATCGTT (SEQ ID NO:147)
Mja	R15	AATTTCAGCC AAATGATCCC ATAAGACTCAA CCGAATACGT CCAGTCCAT CTTCTAACAA (SEQ ID NO:148)
Mja	R16	TCCATTTT CCAATTACCA AATCGATATA ATCTCCCCGC TCCTTAGCAT AGGCATGTAA (SEQ ID NO:149)
Mja	R17	GCGAACTTCA ACAGTTCTC CGTCTTCAT ATCTGCAATA AACTACGAT TGGTTTTGAT (SEQ ID NO:150)

Mja	R18	ATAAATTCCA TCAATCTCTA CAACCCGCT ACCGAAATT GGGCATAAAT AAAGAAAGAG (SEQ ID NO:151)
Mja	R19	CCGACATAAT AAAGTCCAG TCCCATCATC TACAACAAA TCAATCGTA AAATCTCTTC (SEQ ID NO:152)
Mja	R20	TAAAATTCCA TCTTCCACCA TTTCGATAAGT AAGTTCTTT AACTCCTCCC GATTCAATT (SEQ ID NO:153)
Mja	R21	CTCAAACATCT ACATCATAA CGCGACGAAC AACCATTAATT AATTCACTAT TCTCTACTCG (SEQ ID NO:154)
Mja	R22	TTATTCCATT TCCCTCAAGA TAGGAATTTC TTT (SEQ ID NO:155)
Anchor primers		
Mja F		GCTGCCATGG TAGGAGATA TGAACGTTT AAACAAAC (SEQ ID NO:156)
Mja R		GCTCCTCGAG TTATCCATT TCCTCCAAGA TAGG (SEQ ID NO:157)